

Regulatory light chain influences alterations of myosin head induced by actin

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The effect of magnesium-for-calcium exchange and phosphorylation of regulatory light chain (LC₂) on structural organization of rabbit skeletal myosin head was studied by limited tryptic digestion. In the presence of actin, exchange of magnesium bound to LC₂ by calcium in dephosphorylated myosin accelerates the digestion of myosin and heavy meromyosin heavy chain and increases the accumulation of a 50 kDa fragment. This effect is significantly diminished in the case of phosphorylated myosin. Thus, both phosphorylation and cation exchange influences the effect of actin binding on the structural organization of myosin head.

Skeletal muscle myosin; Regulatory light chain; Actin; Limited proteolysis; Myosin phosphorylation

1. INTRODUCTION

It is well established that myosin head internal motion takes place during muscle contraction (for a review see [1–6]). Myosin head flexible internal organization is considered to be based on three mobile, independent folding units of heavy chain and two highly mobile light chains (for a review see [4,5,7]). Many findings provided evidence of conformational changes of myosin head induced by the binding of actin, nucleotide, Ca²⁺ or Mg²⁺ ions [8–22]. It is very probable that actin-activated ATPase is controlled by conformational changes of regulatory light chains induced by Ca²⁺ binding and/or phosphorylation [23,24]. It can be expected that structural alternation of these chains influences internal motions of myosin heads [4,18,25].

The role of regulatory light chains of skeletal muscle myosin remains still unclear, although there exist several findings showing the influence of Ca²⁺ ions and/or phosphorylation on the structure of the light chains in isolated state [26] and in situ [27]. There is evidence that structural changes of regulatory light chain moderate the interaction of myosin head with actin [24,28–31], influence the actin-activated ATPase [32,33] and muscle contraction [34,35]. It has also been reported that myosin heads with intact regulatory light chains exert conformational changes on actin filaments in a Ca²⁺-dependent manner [30,31].

The limited proteolysis method has been the most successful in providing information about structural or-

ganization of myosin head. In the present study this method has been used to obtain information about the influence of the exchange of bound magnesium ions in phosphorylated and dephosphorylated myosin heads for calcium ions on the internal structure of these heads.

2. MATERIALS AND METHODS

2.1. Protein preparation

Phosphorylated and dephosphorylated myosin were obtained by slightly modified method described by Stepkowski et al. [33]. The phosphorylation was performed in the presence of 10 mM pyrophosphate and 20 mM phosphate at pH 7.0. Other compounds remained unchanged. This modification reduces practically completely the probability of dephosphorylation during further steps of purification. The heavy meromyosin (HMM) was obtained according to Stepkowski et al. [29]. F-Actin was purified according to the method of Strzelecka-Golaszewska et al. [36]. Polymerization was performed in the presence of ATP. Protein concentrations were determined by the Biuret method [37] or from the absorbance at 280 nm with absorption coefficients A₂₈₀^{1%} equal to 5.6, 6.49, 11 for myosin, HMM and F-actin, respectively. The *M_r* values were taken as 480 000, 350 000, 42 000 for myosin, HMM and actin, respectively.

2.2. Reagents

Trypsin-TPCK-treated and α-chymotrypsin-TLCK-treated (trypsin-free) were obtained from Sigma Chemical Co., St. Louis. The reagents for electrophoresis were obtained from Serva, Heidelberg. Other reagents used were of analytical grade.

2.3. Digestion procedure

All digestions were performed in 100 mM KCl, 1 mM DTT, 1 mM MgCl₂, 10 mM imidazole, pH 7.0, in 20°C. The myosin and HMM were digested at concentrations of 1 and 2 mg/ml, respectively, in the presence of 1:1 molar ratio of actin-to-myosin head or without actin. EGTA and CaCl₂ were added as described in the legends for figures. Samples of digest (100 μl) were taken at appropriate times and transferred to tubes containing equal vols. of 10% SDS, 10% β-mercaptoethanol, 10 mM Tris-HCl, pH 6.8, and 40% glycerol. Samples were boiled for 3 min and applied to the gel.

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2.4. Electrophoresis and densitometry

Electrophoresis was performed according to Laemmli [38] on slab gels 4.3%/12.5%. Gels were stained with Coomassie blue and after destaining pictures of the gels were taken by a Panasonic CCD video camera and digitized using an IBM 386 computer equipped with Visionetics frame grabber card. The image was then scanned using densitometry software written by Dr. Paweł Pomorski.

3. RESULTS

In our all experiments we have used 1 mM MgCl_2 to have maximal protection of the S1/S2 junction. To assure that we had myosin heads saturated with either magnesium or calcium ions the digestion was performed in the presence of 1 mM MgCl_2 , and 1 mM EGTA or 0.1 mM CaCl_2 . In the latter case, despite a 1 mM concentration of MgCl_2 , the myosin head should have been saturated with Ca^{2+} due to differences in the binding affinity of Ca^{2+} and Mg^{2+} to myosin [39–41]. Myosin with both dephosphorylated or phosphorylated LC_2 have been used in order to check the effect of phosphorylation and Mg^{2+} -for- Ca^{2+} exchange on the conformation of myosin head.

Fig. 1 represents the decrease in the amount of intact myosin heavy chain in the time-course of tryptic digestion of myosin complexes with actin. Heavy chain of dephosphorylated myosin with bound Mg^{2+} after 20 min of tryptic digestion remained undigested in about 50%. When myosin heads were saturated with Ca^{2+} the digestion of heavy chain accelerated. After 20 min of tryptic digestion only about 30% of heavy chain remained undigested (Fig. 1A). For phosphorylated myosin (Fig. 1B) the diminution of intact heavy chain in the course of digestion did not depend on whether Ca^{2+} or Mg^{2+} were bound to myosin head.

The accumulation of a 50 kDa fragment in the time-course of tryptic digestion of dephosphorylated myosin, complexed with actin and saturated with Ca^{2+} , was significantly higher than for that saturated with Mg^{2+} (Fig. 2A). In the case of phosphorylated myosin no significant differences of 50 kDa fragment production was observed (Fig. 2B).

In the absence of actin, the decrease in the amount of intact heavy chain in the time-course of tryptic digestion of dephosphorylated myosin was only slightly dependent on whether myosin was saturated with magnesium or calcium (Fig. 3A). In the case of phosphorylated myosin no significant difference was observed (Fig. 3B).

To get more information about the influence of cation exchange in myosin head on the proteolytic susceptibility of myosin heavy chain, experiments were performed with heavy meromyosin obtained by brief chymotryptic digestion under conditions preserving intact LC_2 [42] in the phosphorylated or dephosphorylated state [29]. Fig. 4 represents electrophoregrams of 20 min digestion patterns (16:1, HMM to trypsin) of dephosphorylated (Fig. 4a–d) and phosphorylated (Fig. 4e–h) heavy meromyosin in the presence of actin and 1 mM

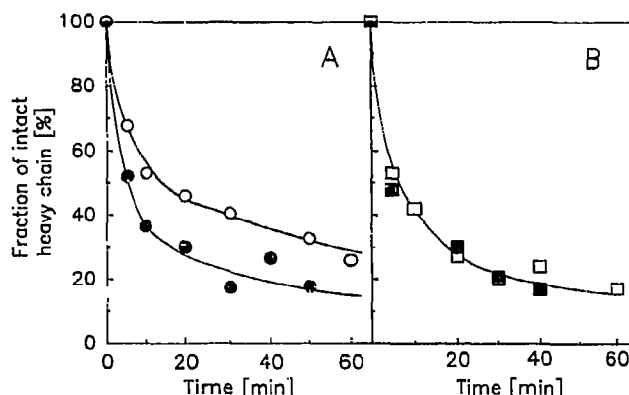


Fig. 1. Time-course of tryptic cleavage of heavy chains myosin in the presence of actin. The digestion was performed at 20°C, enzyme/substrate ratio 1:100 in 0.1 M KCl, 1 mM DTT, 10 mM imidazole buffer, pH 7.0, 1 mM MgCl_2 and either 1 mM EGTA (open symbols) or 0.1 mM CaCl_2 (filled symbols). (A) Dephosphorylated myosin. (B) Phosphorylated myosin. The relative intensity of the heavy chain band was plotted against the time of digestion. Band intensity = 100% at time = 0.

CaCl_2 (Fig. 4a,e), a mixture of 0.1 mM CaCl_2 and 1 mM MgCl_2 (Fig. 4b,f), 1 mM MgCl_2 and 1 mM EGTA (Fig. 4c,g), and 1 mM EDTA (Fig. 4d,h). The pattern in the presence of EDTA differed from the others. There were bands corresponding to 50, 37, 27 and 20 kDa degradation products. The 64 kDa fragment was absent, being totally digested to 37 and 27 kDa fragments, and subsequently to 20 kDa fragments. In our experiment however, the Ca^{2+} and Mg^{2+} bound to myosin head diminished the degradation of the 150 kDa fragment (the heavy chain of heavy meromyosin) and the 64 kDa fragment to a different extent (compare Fig. 4a,b with 4c). Mg^{2+} -saturated myosin head preserves intact 150 kDa peptide more efficiently than Ca^{2+} ; slightly more of 37 kDa fragment was accumulated when the digestion was performed in the presence of Ca^{2+} . The phosphory-

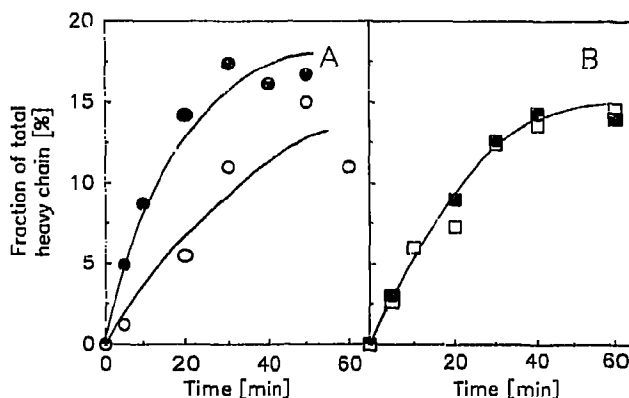


Fig. 2. The accumulation of a 50 kDa myosin fragment plotted against the time of digestion. The conditions and symbols are as in Fig. 1. The relative intensities of the 50 kDa band were calculated assuming the intensity of the heavy chain band at time = 0 as 100%.

lation of LC_2 reduces significantly the differences between the effects of Ca^{2+} and Mg^{2+} ; LC_2 is quickly cut to 18 kDa fragments (LC'_2) and then remains stable.

4. DISCUSSION

It is well known that the protective action of divalent cations on proteolytic cleavage of myosin molecule at the S1/S2 junction is caused by the binding of Mg^{2+} or Ca^{2+} to the LC_2 [9-11]. The calcium ions are significantly more effective than magnesium ions [9,10]. Nearly complete protection of the S1/S2 junction on trypsin cleavage was observed at concentrations of 10^{-7} M Ca^{2+} and 5×10^{-4} M Mg^{2+} [9]. Since in all experiments we have used 1 mM $MgCl_2$ the significant differences we observed in the proteolytic susceptibility, which was dependent on conformational changes of LC_2 or LC'_2 , cannot be explained in connection with protection of the S1/S2 junction. In the present paper, using limited tryptic digestion, it was possible to show that the conformational changes of LC_2 or LC'_2 induced by phosphorylation or by exchange of bound Mg^{2+} for Ca^{2+} influenced the susceptibility of myosin and heavy meromyosin heavy chains for cleavage. The tryptic digestion was chosen by us because in the presence of divalent cations it is known to cut only small fragments from the N-terminal of LC_2 (7 amino acid residues) forming stable LC'_2 with preserved, intact phosphorylation sites and calcium binding domains [18], the presence of which is a necessary condition to study the effects of cation exchange and phosphorylation of myosin light chains while bound to myosin head. Furthermore, removal of these N-terminal residues has little or no effect on actin-induced inhibition of the S1/S2 swivel digestion dependent on the presence of intact LC_2 [18]. In agreement with observations of Miller and Reisler [18] we found that such a shortened light chain is functional in the sense of transmitting conformational changes onto myosin head upon exchange of bound cation and phosphorylation.

It has been well demonstrated that actin protects, in addition to S1/S2 swivel, the 50/20 kDa junction against proteolytic cleavage [13,14,43]. Increased accumulation of the 50 kDa fragment and degradation of heavy chain in the course of digestion of dephosphorylated myosin saturated with calcium ions suggest that the protection by actin of the 50/20 kDa junction is less effective than in the case of magnesium residing in LC_2 . Phosphorylation seems to diminish the effect of Mg^{2+} – Ca^{2+} exchange in LC_2 binding sites on the sensitivity of 50/20 kDa junction for cleavage. Therefore, the protection of 50/20 kDa accompanying binding of actin is influenced by LC_2 .

These findings are in good agreement with those observed by several authors on the influence of LC_2 on actin myosin interaction [24,28,38] and support the postulation of Vibert and Cohen [4] for the possible role of

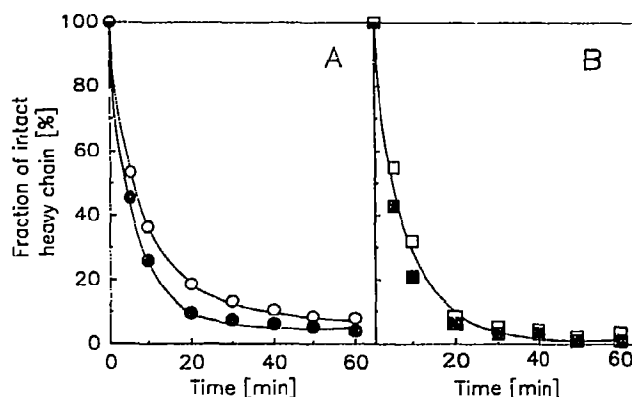


Fig. 3. Time-course of tryptic digestion of dephosphorylated and phosphorylated myosin in the absence of actin. Symbols and conditions are as in Fig. 1.

regulatory light chains in controlling of enzymatic properties of myosin.

According to Shriver and Sykes [44] myosin head can exist in two conformational states in which they are in equilibrium with each other. The transition from one state to another depends on different factors and is influenced by the binding of actin and nucleotides. One can expect that the regulatory light chains sensitive to cation binding and phosphorylation may affect this equilibrium by controlling internal organization of myosin head.

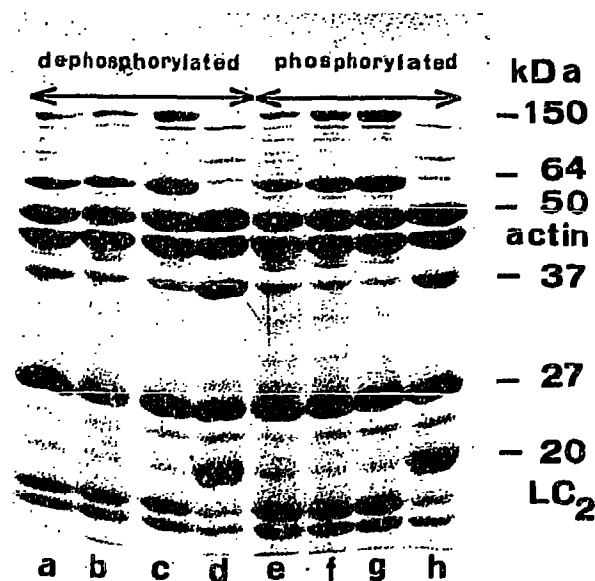


Fig. 4. The electrophoretic pattern of tryptic digestion of HMM in the presence of actin. The HMM has been digested for 20 min at a trypsin/substrate ratio of 1:16 (w/w) at 20°C in 0.1 M KCl, 10 mM imidazole buffer, pH 7.0, 1 mM DTT. HMM with dephosphorylated light chains (lanes a,b,c,d); phosphorylated light chains (lanes e,f,g,h). The samples of HMM were digested in the presence of 1 mM $CaCl_2$ (lanes a,e) or 1 mM $MgCl_2$ and 0.1 mM $CaCl_2$ (lanes b,f) or 1 mM $MgCl_2$ and 1 mM EGTA (lanes c,g) or 1 mM EDTA (lanes d,h).

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